Bonding to caries affected dentine

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Abstract

Objectives: Dentine replacement materials are often placed over caries affected dentine (CAD). The aim of this study was to compare the bonding characteristics and interactions of selected hydraulic calcium silicate-based dentine replacement materials to CAD and sound dentine. Methods: Three hydraulic calcium silicate-based dentine replacement materials were assessed: Biodentine, Retro MTA and Theracal LC. Material characterization was done by scanning electron microscopy and X-ray diffraction analyses. Blocks of sound and CAD were prepared and standardized by Vickers microhardness testing. Half of the affected and sound dentine blocks were pretreated with 5.25% NaOCl prior to material placement. The materials were stored either for 1 week or 24 weeks in 37°C in fully saturated conditions. Shear bond strength was assessed at both time periods. Radiopacity of the interfacial dentine was also evaluated to assess the remineralization potential of the dentine replacement materials. Results: The reaction of Theracal was slower than that of the water-based materials. The bond strengths of different materials did not differ after 1 week ($P>0.05$). The bond strength of Biodentine and Retro MTA increased over time but no change was observed for Theracal. NaOCL pre-treatment deteriorated the bond strength to sound dentine but improvement was observed in affected dentine. Radiopacity changes were observed after 24 weeks. Significance: Biodentine and Retro MTA showed better bonding to CAD. Pretreatment with NaOCl improved the bond strength of dentine replacement materials to CAD.

Keywords	caries; characterization; dentine replacement materials; shear bond strength; demineralized dentine; remineralization

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The data that has been used is confidential

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1st March 2018

Dear Prof Watts

I wood like to submit the manuscript entitled “Bonding to caries affected dentine” to Dental Materials for per review and possible publication.

The work presented has been performed by the named authors. This manuscript is not currently under consideration for publication elsewhere. The authors declare no conflict of interest.

Sincerely,

Josephine (Josette) Camilleri

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Reply to reviewer comments

Comments from the editors and reviewers:
-Reviewer 1

- The study evaluates interactions between three calcium silicate-based dentine replacement materials to sound and caries-affected dentin. The manuscript needs some work with regard to clarity and language. The words "this study clearly shows" was pronounced way too often, the conclusions are clear, it is unnecessary to repeat this many times. Besides, it is a widely accepted fact that bonding to caries-affected dentin is different than sound dentin.
Comment noted and manuscript modified accordingly

The information bars of Figure 1 that show imaging parameters are so pixelated that they are impossible to read. Were the images modified digitally?
The images are original and not modified in any way. I have redone up Figure 1 making sure that when converted to PDF the image bars remain legible

The SEM figures are not clear, they should be better explained to the reader. The 1 week and 24-week images are so different that they are difficult to compare. Were the images taken using different parameters?
The same parameters were used for the imaging. The materials change over a 6 month period. This is very clear from the sem images. Elaborated further the results section

Figure 3 is showing results of a hypomineralized dentine. The manuscript does not include any text about this. Was there any hypomineralization of the already caries affected dentin?
The use of NaOCl and its value to the bonding was mentioned in the literature review. Added to the text that hypomineralized dentine is the pretreaetd one. Thank you for pointing this out

The XRD and spectroscopy results have a lot of information that has not been discussed in a structured manner. If they were not useful for this paper, they should be removed or discussed properly.
Results discussed further

Why was HBSS used? Would artificial saliva give differet results? This should be discussed.
These materials are in contact with dentine thus dentinal fluid. This is why HBSS was used. The dentine replacement materials are not in contact with saliva. Thus using artificial saliva is not relevant to this study.

-Reviewer 2

-
DEMA-2018-175
“Bonding to caries effected dentine”

The main purpose of this manuscript was to evaluate the shear bond strength of 3 different calcium silicate cements on sound and on caries affected dentine ex vivo. Thus the title is too short and does not reflect the aim of the study. The title should be altered to, e.g. "Shear bond strength of three different calcium silicate cements on sound and caries affected dentine ex vivo."
I disagree the title is not informative. The manuscript is about bonding to caries affected dentine and thus the title describes this perfectly. Also short titles are better than long ones.

Introduction
Page 3: "All three of the aforementioned dentine replacement materials are hydraulic calcium silicate-based." That is not true. Whereas Biodentine and Retro MTA are such calcium silicate cements, TheraCal LC is a totally different material. TheraCal LC consists of 45 % composite resin and 45 % Portland cement powder (CEM III) (Gandolfi et al. 2012). Thus, it is a flowable composite resin filled with unset cement powder.
After light-curing TheraCal LC shows a heterogeneous structure, high proportion of large, unhydrogenated particles, Due to the added resin, there is not enough moisture but an incomplete hydration, no reaction of cement content with water, no calcium hydroxide release, only a small amount of calcium ions (see: *Camilleri J*, *Laurent P*, *About I 2014)*. The authors should discuss this point.
We know perfectly well that Theracal has additives and that the hydration mechanisms are different. Section modified to clarify.

Page 3: "Clinically these material[s] are rarely placed on sound dentine." That is not true. Originally, calcium silicate cements were developed to closed iatrogenic defects in the root. In these cases the dentine is not affected by caries.
A succinct review of the pertinent literature is necessary, some publications are simply missing like, e.g. Kaup et al. 2015 determined the shear bond strength of Biodentine to be comparable to a glass ionomer cement.
When used as dentine replacement materials the materials are nearly always placed on caries affected dentine. This was meant for this specific use not for all the other uses of these materials. Section modified for clarity. We have quoted all the relevant literature. There is no literature on bonding to caries affected dentine. On this study we are not evaluating glass ionomers so how Biodentine compared to glass ionomer seems not to be too relevant.

Materials and Methods
Page 4: Please, don´t write that calcium silicate cements are "dentine replacement materials". That's just an advertising slogan of the dental manufacturers.
Dentine replacement is a general term. However it has been removed to avoid unnecessary bias.

How were the teeth stored after extraction? That may influence the shear bond strength. Why were the samples "dried in a vacuum desiccator"? That may influence the results.
Specimens kept in water. The materials were dried in vacuum desiccator as the water interferes with the scanning electron microscopy. This effects results of interphase assessment but not for material testing. It is a routine procedure in fact.

Material characterization: Why were a SEM and XRD analysis performed? The main topic of the manuscript was the shear bond strength. Will this help to explain the results? That remains unclear to me.
The main topic is not only the bond strength but also the material interaction with the caries. Unless materials are characterized we will have no clue on how they hydrate and how they interact with the substrate. This is well explained in the results section.

Assessment of bond strength of the materials to caries affected dentine: How was “caries-affected dentine“ defined? How could you ensure that all teeth were excavated the same way? How could you ensure that in all cavities the same amount of caries was excavated?
Caries affected dentine has been defined in the introduction. We standardized the teeth by microhardness testing to have a baseline. The amount of caries removed is irrelevant. What is relevant is the type of dentine substrate left which was standardized by microhardness.

The use of caries detector dye is not undisputed. Please, discuss.
See comment above. We have standardized out specimens by microhardness testing.

Assessment of radiopacity: Why was that done? This remains also unclear to me. Please, explain. Would it not been better to evaluate the dentine – cement interface with SEM and XRD analysis to explain the results of the shear bond test?
The radiographic assessment was performed to assess the remineralization potential. The interphase is not the main scope of this research. We are looking at remineralization potential and investigated this by bond strength and radiographic assessment.

Results
Detailed results of the fracture mode is missing. E.g. a table or pictures may be helpful.
Added to manuscript

Page 13: “[23, 24] (22, 23)” Which citation is correct?

References
Ref. 10: avoid manufacturer’s information as reference.
Ref. 12 + 25: incomplete
Noted and modified

Please, stay to one spelling “dentine” or “dentin”.
Noted and modified

Bonding to caries affected dentine

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Key words: caries; characterization; dentine replacement materials; shear bond strength; demineralized dentine; remineralization

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Bonding to caries affected dentine

Abstract

Objectives: Dentine replacement materials are often placed over caries affected dentine (CAD). The aim of this study was to compare the bonding characteristics and interactions of selected hydraulic calcium silicate-based dentine replacement materials to CAD and sound dentine.

Methods: Three hydraulic calcium silicate-based dentine replacement materials were assessed: Biodentine, Retro MTA and Theracal LC. Material characterization was done by scanning electron microscopy and X-ray diffraction analyses. Blocks of sound and CAD were prepared and standardized by Vickers microhardness testing. Half of the affected and sound dentine blocks were pretreated with 5.25% NaOCl prior to material placement. The materials were stored either for 1 week or 24 weeks in 37℃ in fully saturated conditions. Shear bond strength was assessed at both time periods. Radiopacity of the interfacial dentine was also evaluated to assess the remineralization potential of the dentine replacement materials.

Results: The reaction of Theracal was slower than that of the water-based materials. The bond strengths of different materials did not differ after 1 week ($P>0.05$). The bond strength of Biodentine and Retro MTA increased over time but no change was observed for Theracal. NaOCl pre-treatment deteriorated the bond strength to sound dentine but improvement was observed in affected dentine. Radiopacity changes were observed after 24 weeks.

Significance: Biodentine and Retro MTA showed better bonding to CAD. Pretreatment with NaOCl improved the bond strength of dentine replacement materials to CAD.

Key words: caries; characterization; dentine replacement materials; shear bond strength; demineralized dentine; remineralization

Highlights:

- The bond strength of evaluated dentine replacement materials to dentine did not defer after 1 week.
- The bond strength of Biodentine and Retro MTA increased over time but no change was observed for Theracal
- NaOCl pre-treatment deteriorated the bond strength to sound dentine but improvement was observed in affected dentine.

1. Introduction

Carious dentin consists of two distinct layers: an outer bacterially infected layer of dentine, and an inner layer of affected dentine [1]. The caries-infected layer (CID) is highly demineralized, physiologically unremineralizable and contains irreversibly denatured collagen fibrils with a virtual disappearance of cross-linkages [2, 3]. On the other hand, the caries-affected layer (CAD) is uninfected, partially demineralized and physiologically remineralizable [2-4]. High porosity and exposure of collagen fibers along with a decrease in the surface energy are seen in the inter-tubular CAD [2-4]. Collagen cross-linking remains intact which can function as a scaffold for remineralization of intertubular dentine [2-4]. Therefore, the caries-affected layer should be preserved during clinical treatments.

It has been shown that CAD exhibits lower bond strengths to restorative materials such as glass ionomer and composite resins than sound dentine [5, 6]. The change in chemical and morphological characteristics of CAD may be a reason for this lower bond strength. As CAD is partially demineralized, an irregular and thicker hybrid layer enriched with organic components is created on it [7-9].

In an attempt to improve bond strength to CAD, studies have suggested pretreatment of dentine with sodium hypochlorite solution (NaOCl) [8]. NaOCl can effectively dissolve organic components. Taniguchi et al. [8] demonstrated that pretreatment with 6% NaOCl for

15 s could significantly improve the bond strengths of both 1-step and 2-step self-etch system to CAD. This finding was attributed to the dissolution of superficial organic components of smear layer by NaOCl.

When treating deep carious lesions, the dentine replacement materials come in contact with CAD. Hydraulic calcium silicate cements have shown a lot of promise as dentine replacements due to their beneficial effect on the pulp. Biodentine (Septodont, Saint Maur des Fosses, France) [10] and Retro MTA (BioMTA, Seoul, Korea) [11] are fast setting dentine replacement materials exhibiting clinically suitable properties. Theracal LC (Bisco, Schaumburg, IL, USA) is a resin-modified light curable dentine replacement material used as a liner under composite restorations [12]. It contains a special hydrophilic resin matrix, which allows water penetration and ion release, resulting in apatite formation and sealing of the tooth [13]. The Biodentine and Retro MTA are water-based and composed mostly of tricalcium silicate cement. The Theracal is resin based and contains fillers and other additives. Although previous research has reported the apatite formation and the ion release in solution [13], the calcium ion releasing ability and hydration of the tricalcium silicate component of the Theracal is disputed [14, 15]. This will affect the material clinical performance.

Most research on the interactions between hydraulic calcium silicate-based dentine replacement materials and dentine is performed using sound dentine [16, 17]. Clinically, when used over the pulp, these material are rarely placed on sound dentine. Therefore, the aim of this study was to compare the bonding characteristics and interactions of hydraulic calcium silicate based dentine replacement materials to CAD and sound dentine. The null hypothesis was that the bond of these material to CAD does not differ from sound dentine and pretreatment of dentine with NaOCl does affect their bond strength.

2. Materials and methods

Three hydraulic calcium silicate-based materials were assessed. These included:

- Biodentine (Septodont, Saint Maur des Fosses, France)

- Retro MTA (BioMTA, Seoul, Korea)
- Theracal LC (Bisco, Schaumburg, IL, USA).

The materials were prepared according to manufacturer's instructions. Once set, they were placed in Hank's balanced salt solution (HBSS, H6648, Sigma Aldrich, St. Louis, MO, USA) for 1 week and 24 weeks time periods. At each time point the set material were removed from solution and were dried in a vacuum desiccator and tested.

2.1. Material characterization

Cylindrical specimens 10 mm in diameter and 2 mm high were prepared. At each time point the specimens were removed from solution, dried and prepared for characterization. Three replicates were prepared for each test. The three materials were characterized by using scanning electron microscopy (SEM) and X-ray diffraction (XRD) analysis. For scanning electron microscopy the dried specimens were impregnated in resin (Epoxyfix, Struers GmbH, Ballerup, Denmark) under vacuum. The resin blocks were then ground with progressively finer diamond discs and pastes using an automatic polishing machine (Tegramin 20, Struers GmbH, Ballerup, Denmark). The specimens were mounted on aluminum stubs, carbon coated and viewed under the scanning electron microscope (Zeiss MERLIN Field Emission SEM, Carl Zeiss NTS GmbH, Oberkochen, Germany). Scanning electron micrographs of the different material microstructural components at different magnifications in back-scatter electron mode for the polished samples were captured and energy dispersive spectroscopy (EDS) was also performed.

For phase analysis the dried materials were crushed to a fine powder using an agate mortar and pestle. Phase analysis of the powders was performed using a Bruker D8 diffractometer (Bruker Corp., Billerica, MA, USA) with Co Kα radiation (1.78A˚). The X-ray patterns were acquired in the 2θ (15–45°) with a step of 0.02° and 0.6 seconds per step.

Phase identification was accomplished using a search-match software utilizing ICDD database (International Centre for Diffraction Data, Newtown Square, PA, USA).

2.2. Assessment of bond strength of the materials to caries affected dentine

Human posterior teeth were used for this experiment. They were extracted for various reasons and were kept in water until use. Two types of dentine blocks (60 each) were prepared. One set was from sound dentine and the other from caries affected dentine. For sound dentine caries free teeth were selected, the enamel was trimmed from the surface of teeth with a gypsum model trimmer to achieve a flat dentine surface. For carious dentine specimens, molar teeth with class 1 occlusal carious lesions were selected. To expose the caries-affected dentine the enamel portion was trimmed in a gypsum model trimmer to allow direct access to the carious dentine. The carious lesions in dentine were then removed with a manual excavator assisted by a caries detector dye (Seek, Ultradent, USA). Reaching the hard dentine layer was verified by dentine resistance to excavation, color and visual inspection. Both caries-free and caries-affected dentine specimens were standardized by assessment of the surface hardness evaluated by Vickers microhardness testing (Bareiss Prufgeratebau GmbH, Oberdischingen, Germany). Caries affected dentinal blocks with an average microhardness of 50-55HV were selected. Sound dentine specimens were standardized by microhardness values (100-105 HV) [18].

All specimens (sound and affected dentine) were mounted in acrylic resin. Half of the affected and sound dentine blocks were pretreated with 5mL of 5.25% NaOCl prior to material placement. After pretreatment with NaOCl and before material placement specimens were finally rinsed with normal saline. The microstructure of the affected and sound dentine was evaluated by scanning electron microscopy before and after pretreatment with NaOCl to evaluate the effect of the pretreatment on the dentine microstructure. The dentine specimens that were pretreated with NaOCl are referred to as hypomineralized dentine. Stereomicroscopic images (Olympus SZ30, Olympus, Japan) of the dentine before and after pretreatment were also captured.

A polyvinyl mold (3.5 mm diameter and 3 mm height) was placed over the middle of the flattened dentinal surface of blocks. Biodentine and Retro MTA were prepared according to the manufacturer's instructions. Theracal LC was light cured for 20 seconds at 600 mW/ cm2 using an LED light source. Moulds were removed after (15 minutes) confirming the initial set of the cement. All specimens were then be incubated at 37℃ in fully saturated conditions. Half of the specimens of each subgroup were incubated for 1 week and the other half were incubated for 24 weeks.

Shear bond strength assessment was performed after incubation by a universal testing machine (Z050, Zwick/Roell, Ulm, Germany) with a chisel-edge plunger edge aimed at the dentine replacement material/adhesive interface with a speed of 0.5 mm/ min. Shear bond strength in Nmm-2 was calculated by dividing the peak load at failure by the specimen surface area. Failure modes were evaluated by a single operator under a stereomicroscope (Olympus SZ30, Olympus, Japan) at ×20 magnification. Failure modes were categorized in one of the following groups:

Mode 1. Adhesive failure that occurred at the filling material and dentine interface

Mode 2. Cohesive failure within the filling material

Mode 3. Mixed failure mode

2.3. Assessment of radiopacity

The dentinal blocks were placed on a digital sensor (RVG 5000 Kodak; Eastman Kodak Company, Vincennes, France) and fixed with wax in a way that the dentine surface in which would come in contact with dentine replacement materials and was going to be evaluated was perpendicular to the sensor's surface. All specimens were placed at a 30 cm distance for 0.32 s in a dental X-ray unit (70 kVp/7 mA) connected to a digital system (Kodak RVG 5000 Trophy for Eastman Kodak Company). The X-ray unit was kept in the same position throughout the experiment. The radiographic images were saved in JPEG format

and stored directly on a computer connected to the digital system through its software (KDIS 6.8 Patient File; Eastman Kodak Company Digital analysis procedures). The same procedure was done on the specimens after shear bond testing. The radio-opacity (in pixels) of the surface of each sample was determined in the digital radiographs by densitometry analysis of the Kodak software. The average opacity of the surface was provided by the software.

2.4 Statistical analyses

The data were evaluated using Statistical Package for the Social Sciences software (PASW Statistics 18; SPSS Inc, Chicago, IL). One-way analysis of variance and Tukey post-hoc tests at a significance level of P = 0.05 were used to perform multiple comparison tests.

3. Results

3.1 Material characterization

The scanning electron micrographs and energy dispersive spectroscopic plots of the three test materials are shown in Figures 1a and 1b. The XRD scans are shown in Figure 2. From the scanning electron micrographs it was evident that Biodentine matured faster than the Retro MTA but by 24 weeks the Retro MTA also exhibited reaction rims around the cement particles from the hydration process. After 1 week immersion in HBSS the cement particles of Theracal did not show any signs of hydration. Reaction rims were observed after 24 weeks of

immersion but the reaction rims were still very discreet. The EDS analyses for all materials showed strong peaks for calcium, silicon and oxygen as part of the tricalcium silicate cement. Both Retro MTA and Biodentine showed peaks for zirconium. Both Retro MTA and Theracal exhibited a peak for aluminium indicating the presence of an aluminium rich phase in the cement. The Theracal showed a strontium peak on EDS analyses but this was not evident in the XRD scan thus indicating the glassy amorphous phase. No changes in the EDS analyses was observed at the different time periods tested.

The XRD scans also show the similar maturation for both Biodentine and Retro MTA at 24 weeks with the formation of calcium hydroxide and obliteration of the tricalcium silicate peaks at 32 degrees showing their hydration. The slower reaction of the Theracal was evident even in the XRD scans as at both 1 week and 24 weeks no calcium hydroxide peaks were visible. Some reaction of the tricalcium silicate was evident as there was a reduction in peak height of this phase at 32 and 34 degrees after 24 weeks of immersion in solution (Figure 2).

3.2. Shear bond strength

The results of shear bond strength of the pulp capping materials to different types of dentine substrate after 1 and 24 weeks is shown in Figure 3a. There was no difference in the bond strengths of the different materials to the dentine ($P > 0.05$) after 1 week. The bond strengths of Retro MTA to intact dentine was higher than to intact dentine pretreated with NaOCl and affected dentine ($P = 0.049$, $P = 0.021$ respectively). Theracal also exhibited deterioration in bond strength when bonding to intact dentine pretreated with NaOCl and affected dentine ($P < 0.001$, $P = 0.003$ respectively) but not to affected dentine pretreated with

NaOCl ($P = 0.075$). Biodentine demonstrated similar bond strengths on all dentine types ($P > 0.05$).

After 24 weeks there was a difference in the bond strengths on intact dentine for all materials tested with Retro MTA and Biodentine showing higher bond strengths after maturation ($P < 0.001$). No change was observed for Theracal where values remained stable with no improvement in bond strengths with maturation ($P > 0.05$). No changes were observed on pretreated hypomineralized dentine for all materials where shear bond strength values were every low at both time frames.

The Retro MTA and Biodentine exhibited a higher bond strength after 24 weeks on affected dentine ($P < 0.001$) but no change was observed in Theracal over the 24 week period ($P = 1$). When the affected dentine was pre-treated, the bond strengths improved and this was enhanced with increasing material maturity ($P < 0.001$).

The bond strengths were effected by material maturation in Biodentine and Retro MTA and also by the pretreatment with sodium hypochlorite. In sound dentine the pre-treatment deteriorated the bond strength but an improvement was observed in affected dentine. The failure mode was mostly mixed for all material tested on all dentine substrates. The different types of failure modes are shown in Figure 3b. A large number of Theracal specimens detached prior to testing after being subjected to 6 months immersion in fluids.

The electron micrographs of the dentine blocks are seen in Figure 4a showing the different microstructure of the dentine. Surface deposits were evident in intact dentine and the deposits increased after pre-treatment with sodium hypochlorite. Less surface deposits were present in affected dentine but sodium hypochlorite application increased the intensity of these deposits even in affected dentine. The stereo micrographs exhibited a change in colour for the affected dentine when pre-treated with sodium hypochlorite as indicated in Figure 4b.

3.3. Changes in radiopacity of dentine blocks

The changes in radiopacity after 1 week and after 24 weeks of application of the materials on the different dentine substrates is shown in Figure 5. All the materials exhibited a similar radiopacity after 1 week of application. There was no change in radiopacity of the dentine after 1 week assessment. After 24 weeks the Retro MTA and Theracal exhibited a change in radiopacity of intact dentine ($P < 0.001$). The hypomineralized intact dentine and both types of affected dentine was modified by all the materials exhibiting a rise in radiopacity after 24 weeks exposure ($P < 0.001$).

4. Discussion

In the current study the bonding characteristics and interactions of a number of dentine replacement materials was investigated. The materials were chosen on purpose as they are all based on hydraulic calcium silicate but they had different chemistries. The Retro MTA is basic mineral trioxide aggregate formulation with no additives to enhance its characteristics. Biodentine is a new generation material which includes additives and thus enhanced calcium ion release [14, 15, 19]. Theracal is resin based and thus releases less calcium [14, 15]. The materials were characterized to show the specific material features. Although all the materials had a tricalcium silicate phase they showed different hydration characteristics which could account for their diverse behavior in contact with dentine. The Biodentine and Retro MTA were both water-based but due to its additives, Biodentine reacted faster and produced calcium hydroxide earlier than Retro MTA. This was evident from the reaction rims shown in the electron micrographs and the calcium hydroxide peaks shown in the XRD scans. The long term formation of calcium hydroxide had more effect on the remineralization and also the long term bond strength thus Retro MTA exhibited better bond strengths and more remineralization than Biodentine. Theracal, which is resin based and exhibited very slow hydration and no formation of crystalline calcium hydroxide showed poor bonding.

The null hypothesis of this investigation was rejected as difference was seen in the shear bond strength of the evaluated dentine replacement materials to sound and CAD. Also,

in sound dentine the pre-treatment with NaOCl, deteriorated the bond strength but an improvement was observed in CAD. In clinical cases of pulp capping these dentine replacement materials come in contact with CAD. CAD is partially demineralized and therefore more porous and softer than sound dentine, has narrow and obliterated dentinal tubules due to deposition of intratubular dentine and has a hybrid layer thicker than that of sound dentine [2-4]. Due to these differences, the bonding characteristics and interactions between dentine replacement materials and CAD may differ from sound dentine. Therefore, this evaluation is of clinical relevance. Many studies have shown that the bond strength values of restorative materials to sound dentine is higher than that of CAD [5, 6]. In the current study, the pretreatment of affected dentine with NaOCl, improved the material bond strength as in previous literature [8]. It is important to have good marginal seal and better bond strength for the longevity of and success of pulp capping. Better bonding to dentine reduces marginal leakage and subsequently reduced the incidence of secondary caries, post-operative sensitivity and pulpal pathology [20].

The bond strength of Biodentine and Retro MTA increased over time. This is due to progression in hydration and maturation [21] of these cements as seen in XRD analysis. At 24 weeks peaks for calcium hydroxide were present and the tricalcium silicate peaks disappeared. With progression in maturation and hydration calcium hydroxide crystalline structures are formed due to saturation of the soluble form [22]. The apposition of crystalline structures may improve the mechanical bond between these cements and dentine. No change was observed in bond strength values of Theracal over time. This may be due to its compromised hydration and maturation. According to XRD evaluations no calcium hydroxide peaks were visible on the XRD plots at 24 weeks but some reaction of the tricalcium silicate was evident. Contrary to Biodentine and Retro MTA, Theracal does not include water in its

formulation and therefore, this material's hydration completely depends on the water taken up from the environment and its diffusion within the material [15]. Thus, the hydration of this material may be compromised when compared with water-based materials such as Biodentine as seen in this study.

The bonding mechanism of Biodentine to intact dentine has already been postulated. It was shown that transfer of calcium ions occurs across the tooth to material interface creating a mineral infiltration zone [16]. This hypothesis was discredited by a further study where no mineral transfer was shown at the interface [17].

In sound dentine the pre-treatment deteriorated the bond strength but an improvement was observed in affected dentine. The microstructure of caries affected dentine and sound dentine is different as shown in the scanning electron micrographs. The pretreatment with sodium hypochlorite further changed the dentine microstructure. This pattern was also seen in the case of bonding to restorative materials [8]. Improvement of bond strength to CAD after pretreatment with NaOCl was expected as it causes dissolution of superficial organic components of smear layer in this type of dentine therefore allowing better contact between the cements and CAD.

Over time the radiopacity of intact dentine increased in specimens in contact with Retro MTA and Theracal. This may be due to either remineralization of dentine over the 24 week period or migration of elements [23] such as zirconium or aluminum from dentine replacement materials to dentine. In the case of Retro MTA as progression in hydration and maturation was seen, remineralization of dentine can be considered. But in the case of Theracal as its hydration was compromised migration of elements may be a more probable cause for the increase in opacity. This is in agreement with recent studies where remineralization was observed in dentine following the placement of hydraulic calcium silicate-based dentine

replacement materials [24, 25]. In one of the studies intact dentine was used and calcium ion transfer was shown to happen together with the formation of calcium phosphate at the interface [25]. In the other study [24] the dentine was demineralized with formic acid. Although this is valid in vitro, formic acid can affect the hydration of hydraulic calcium silicate-based materials [26] thus is not suitable to use on dentine as traces of it may affect the hydration thus the remineralization process. Further research is required to evaluate the mechanisms of remineralization and bonding mechanisms of dentine replacement materials to caries affected dentine. The clinical relevance of this is very clear. Studies of material bond strengths to sound dentine are of limited value clinically as verified in this study.

5. Conclusion

Biodentine and Retro MTA showed better bonding to CAD. Pretreatment of CAD with NaOCl improved the bond strength of silicate-based dentine replacement materials to CAD.

6. Acknowledgements

Ing James Camilleri of the Department of Metallurgy and Materials Engineering for his technical expertise. ERDF (Malta) for the financing of the testing equipment through the project: "Developing an Interdisciplinary Material Testing and Rapid Prototyping R&D Facility" (Ref. no. 012).

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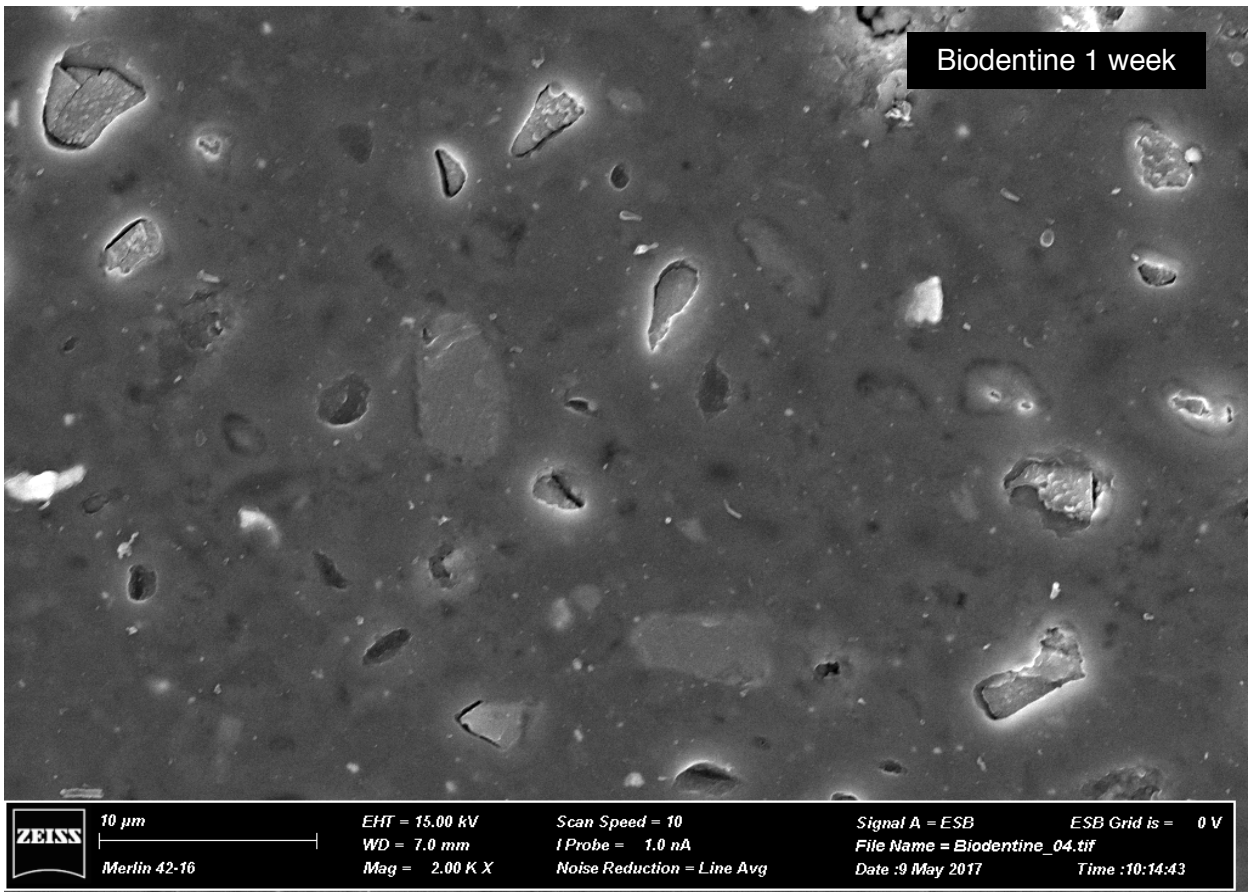
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Merlin 42-16
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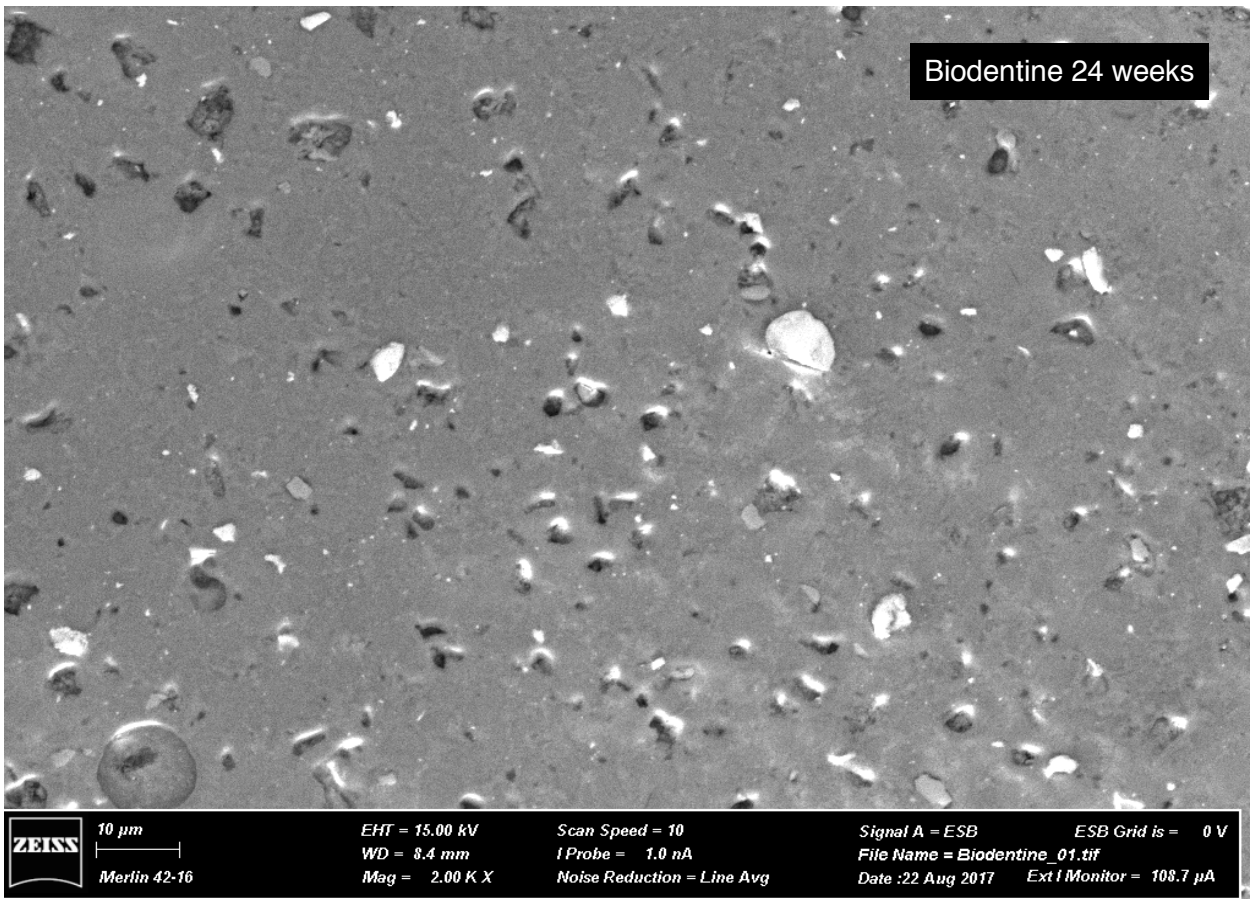
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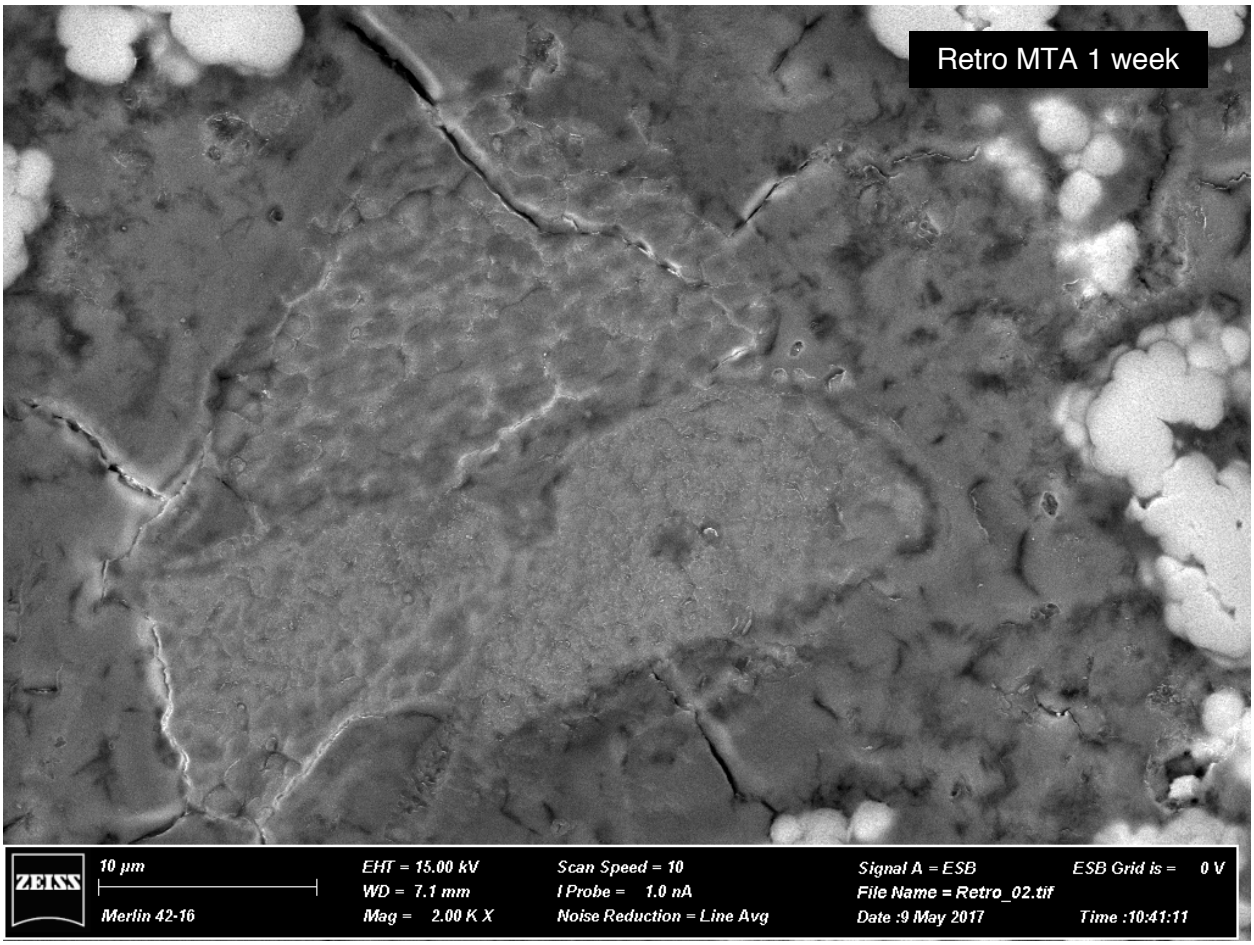
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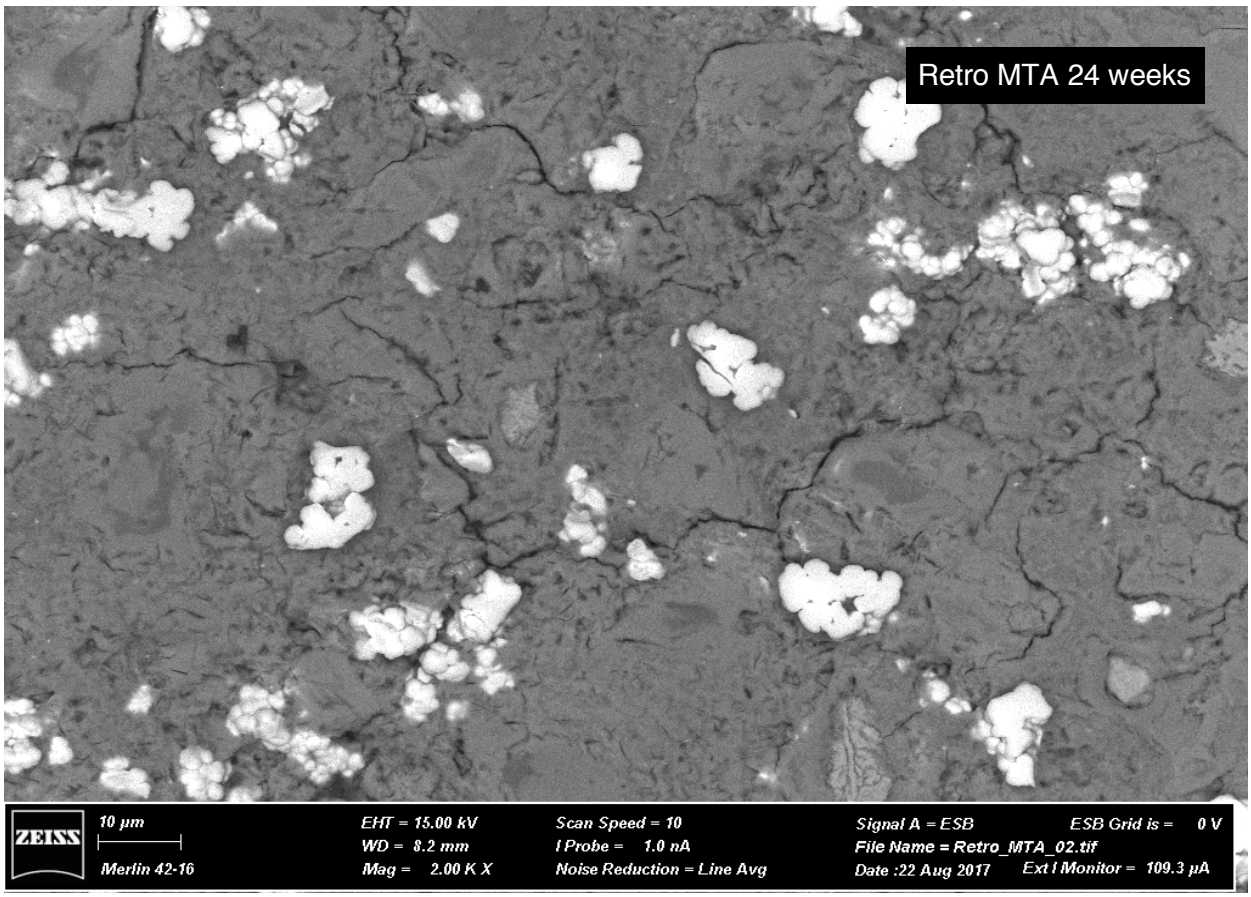
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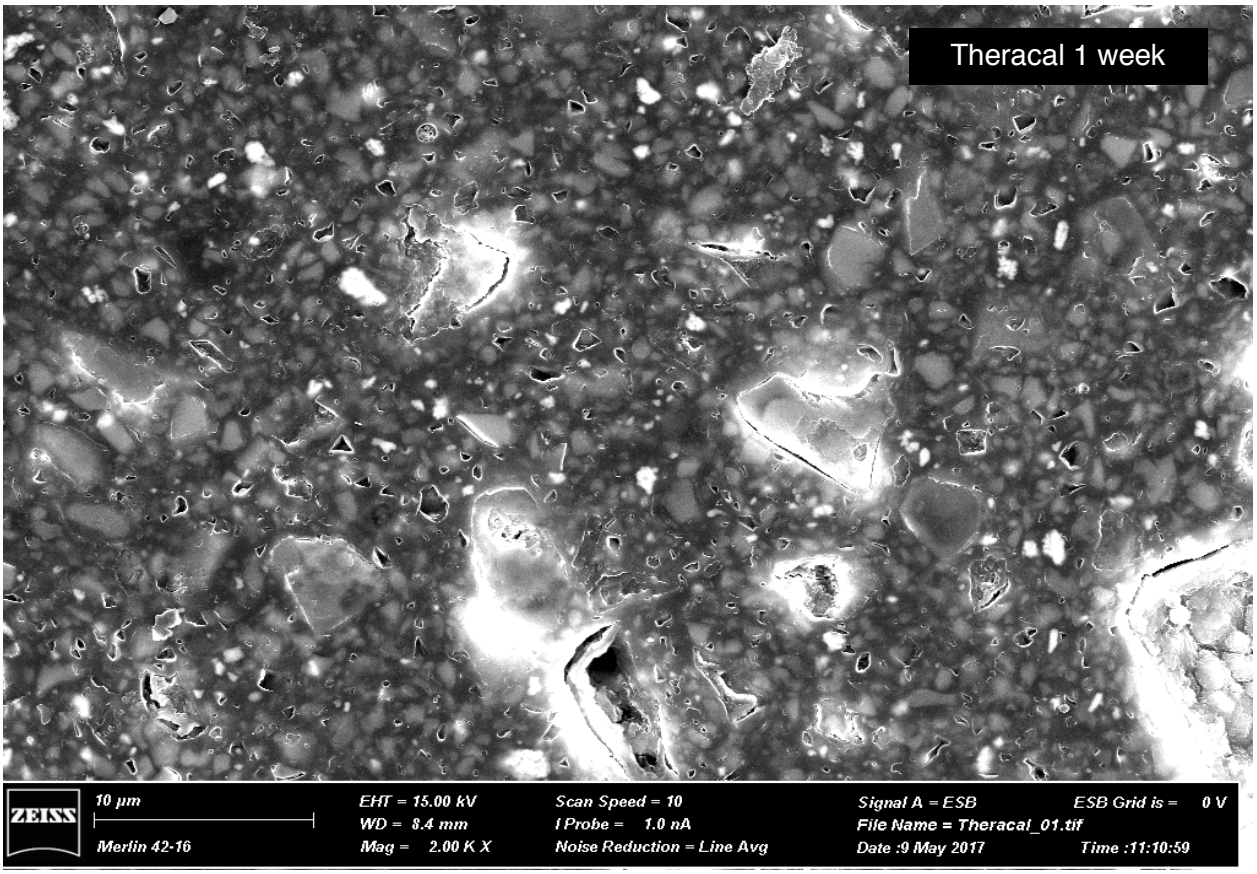


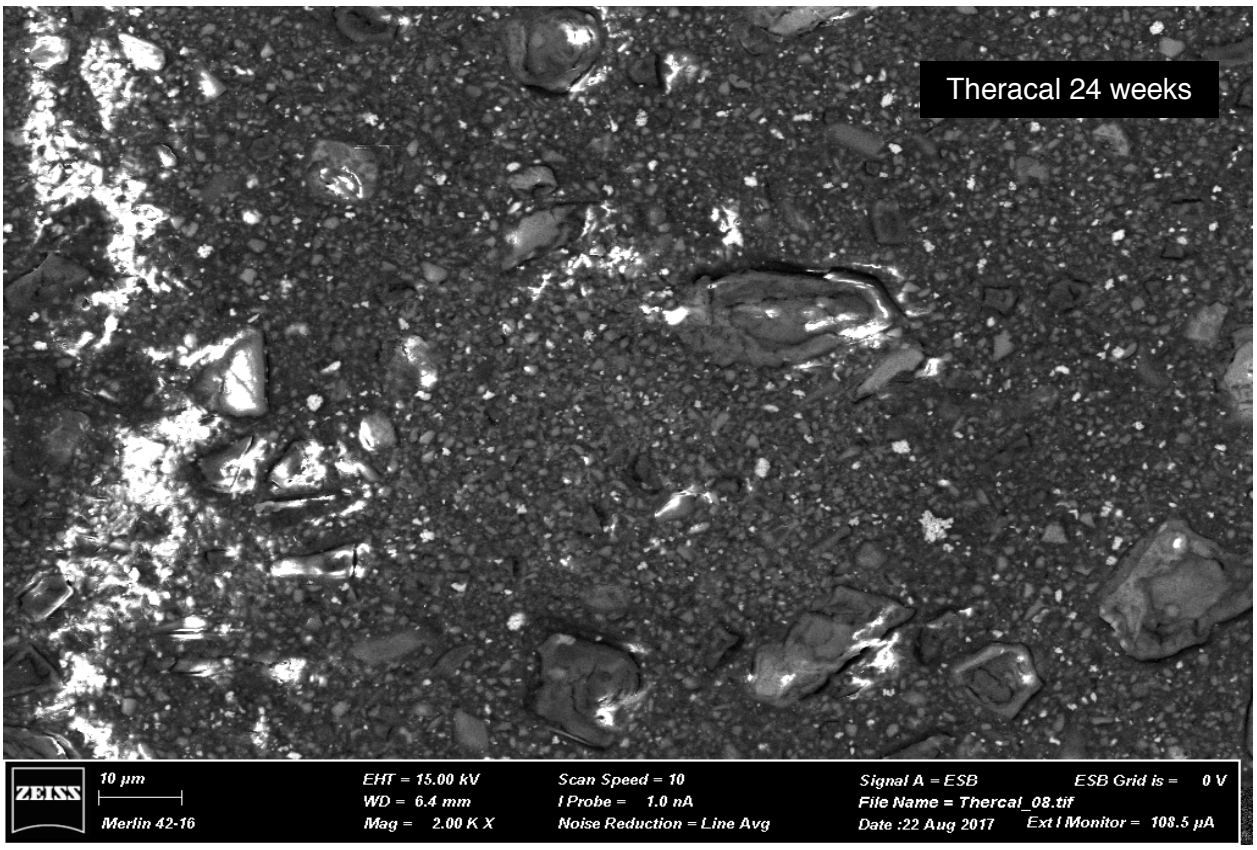
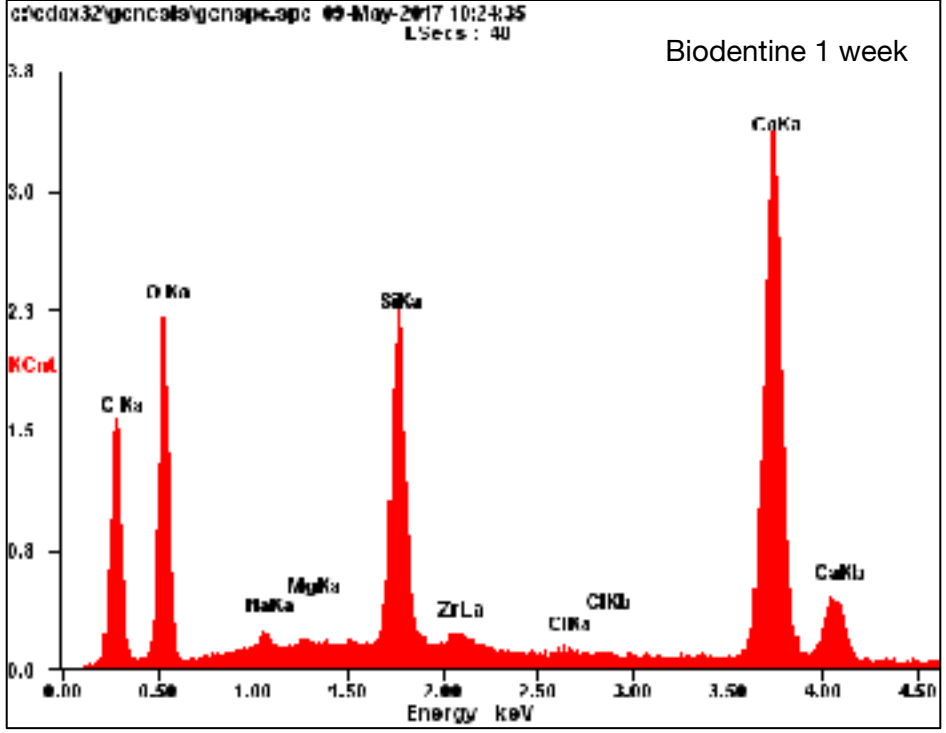


Figure 1a: Back scatter scanning electron micrographs of the test materials after 1 week and 24 weeks immersion in Hank's balanced salt solution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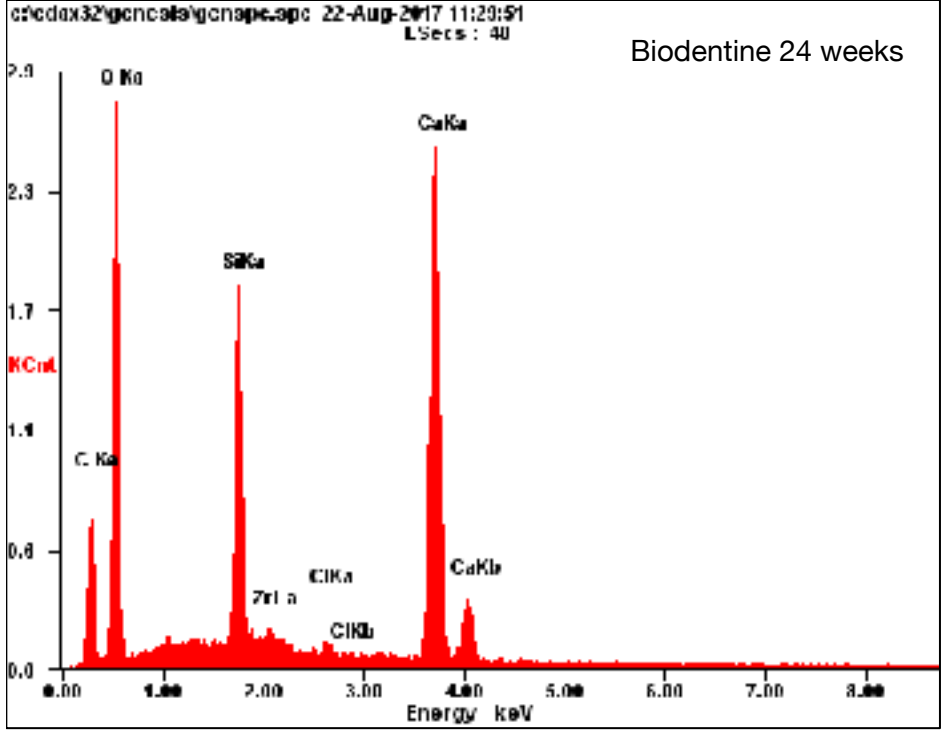
O Ka
C Ka
SiKa
CaKa
MgKa
NaKa
ZrLa
ClKa
ClKb
CaKb
Energy keV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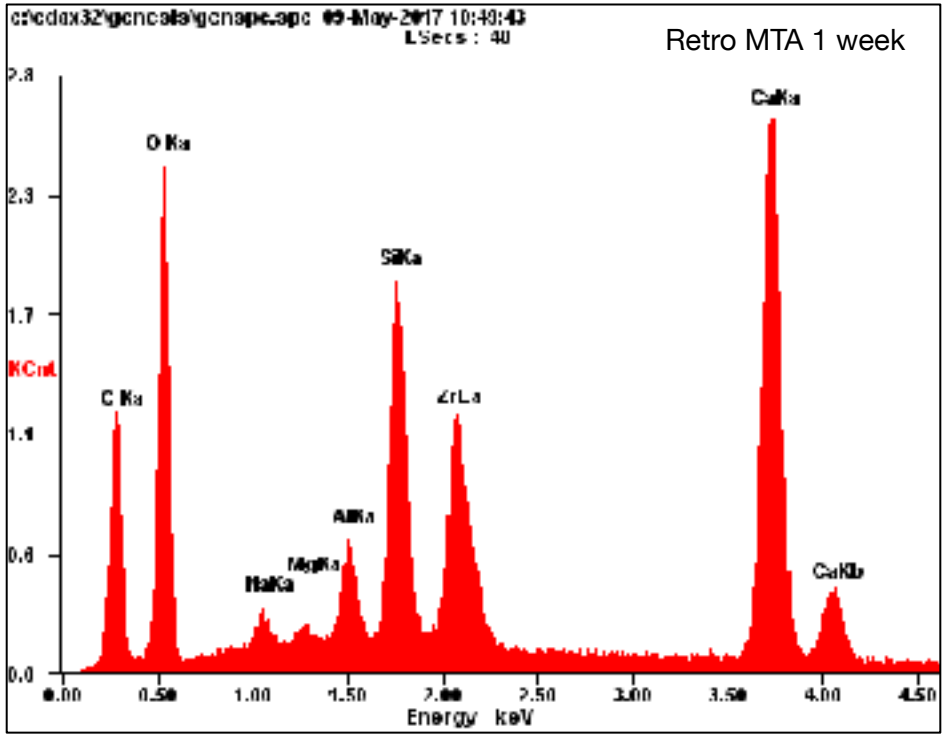
C Ka
SiKa
CaKa
ZrLa
ClKa
ClKb
CaKb
Energy keV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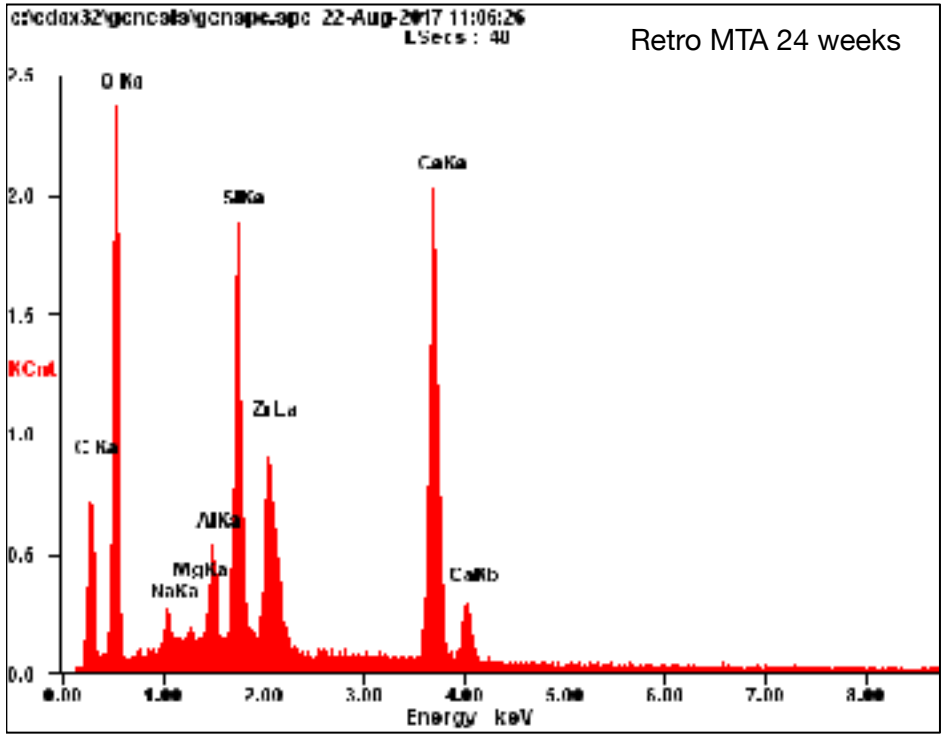
O Ka
C Ka
SiKa
CaKa
ZrLa
AlKa
MgKa
NaKa
CaKb
Energy keV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Energy keV

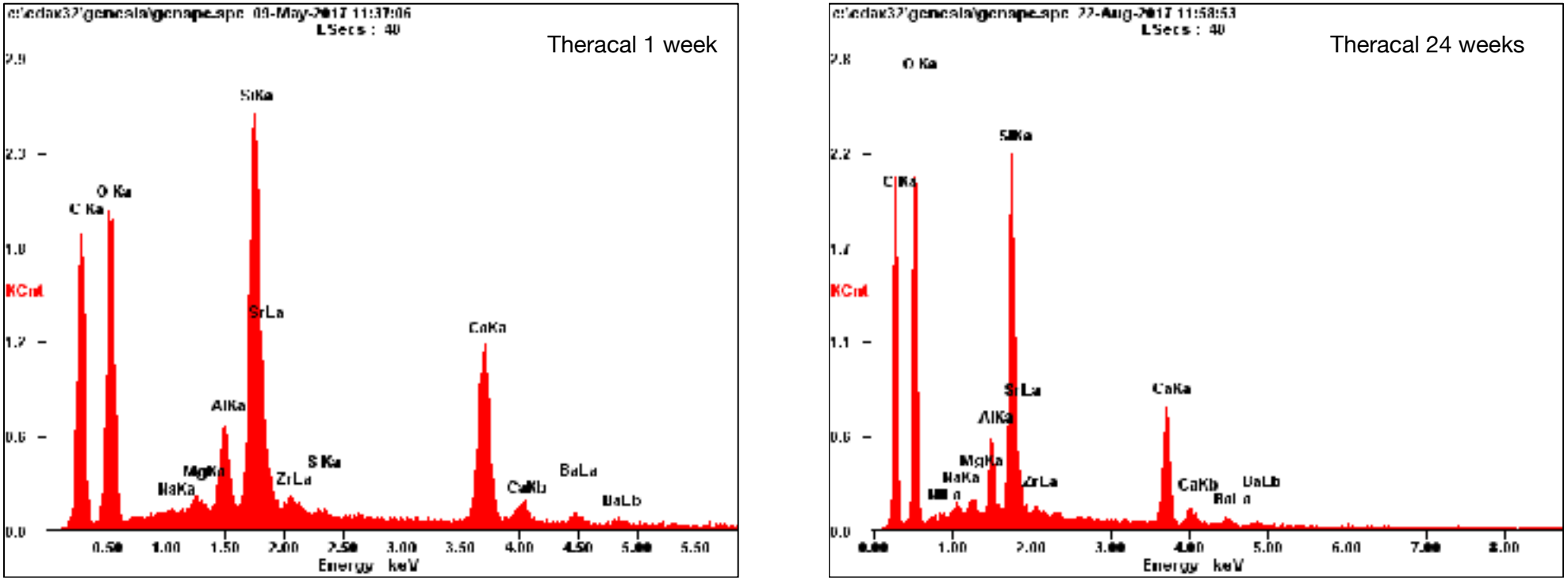


Figure 1b: Energy dispersive spectroscopy plots of the test materials after 1 week and 24 weeks immersion in Hank's balanced salt solution

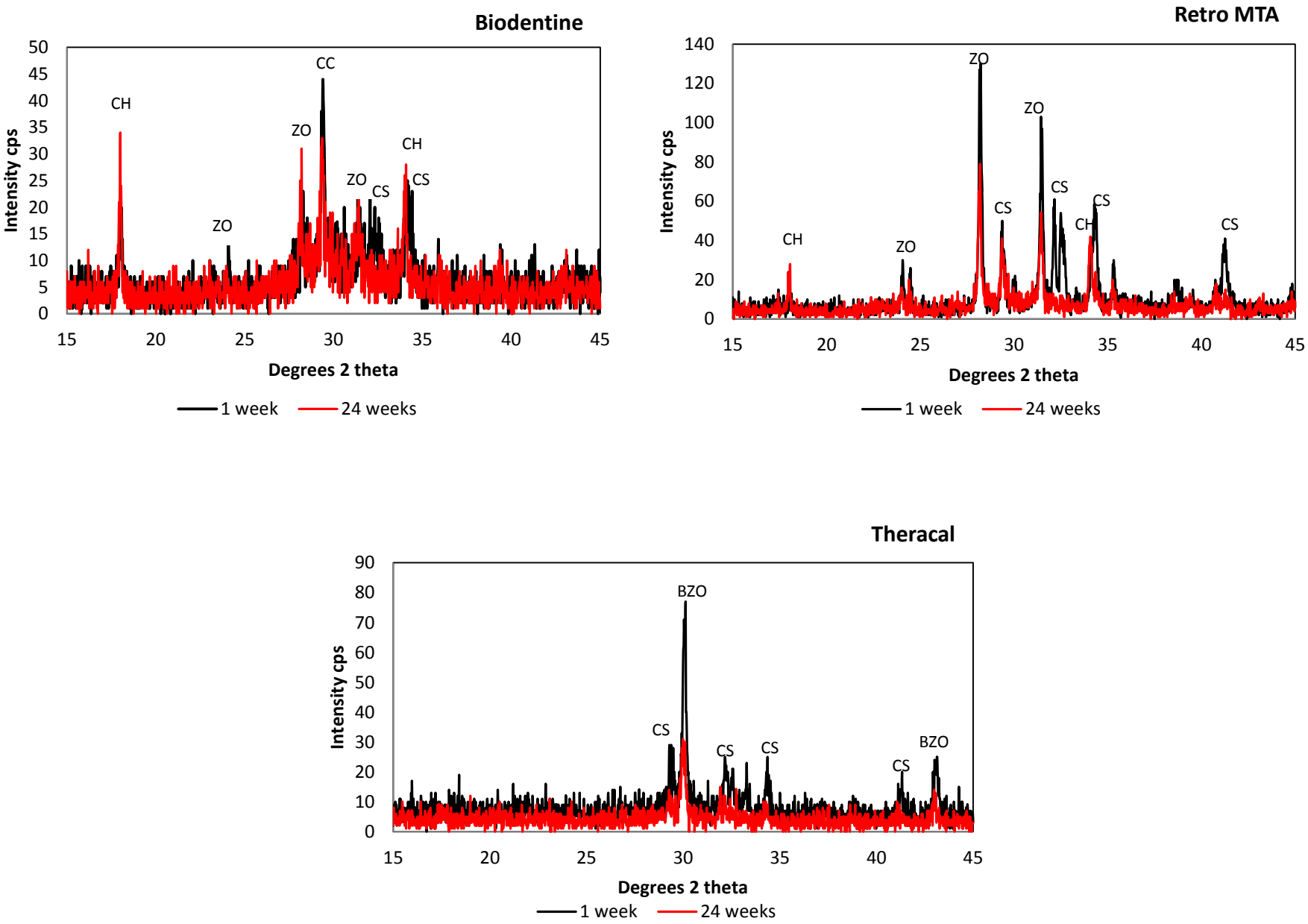


Figure 2: X-ray diffraction scans of the test materials after immersion in HBSS for 1 and 24 weeks (BZO: barium zirconate; CC calcium carbonate; CH: calcium hydroxide; CS tricalcium silicate; ZO: zirconium oxide)

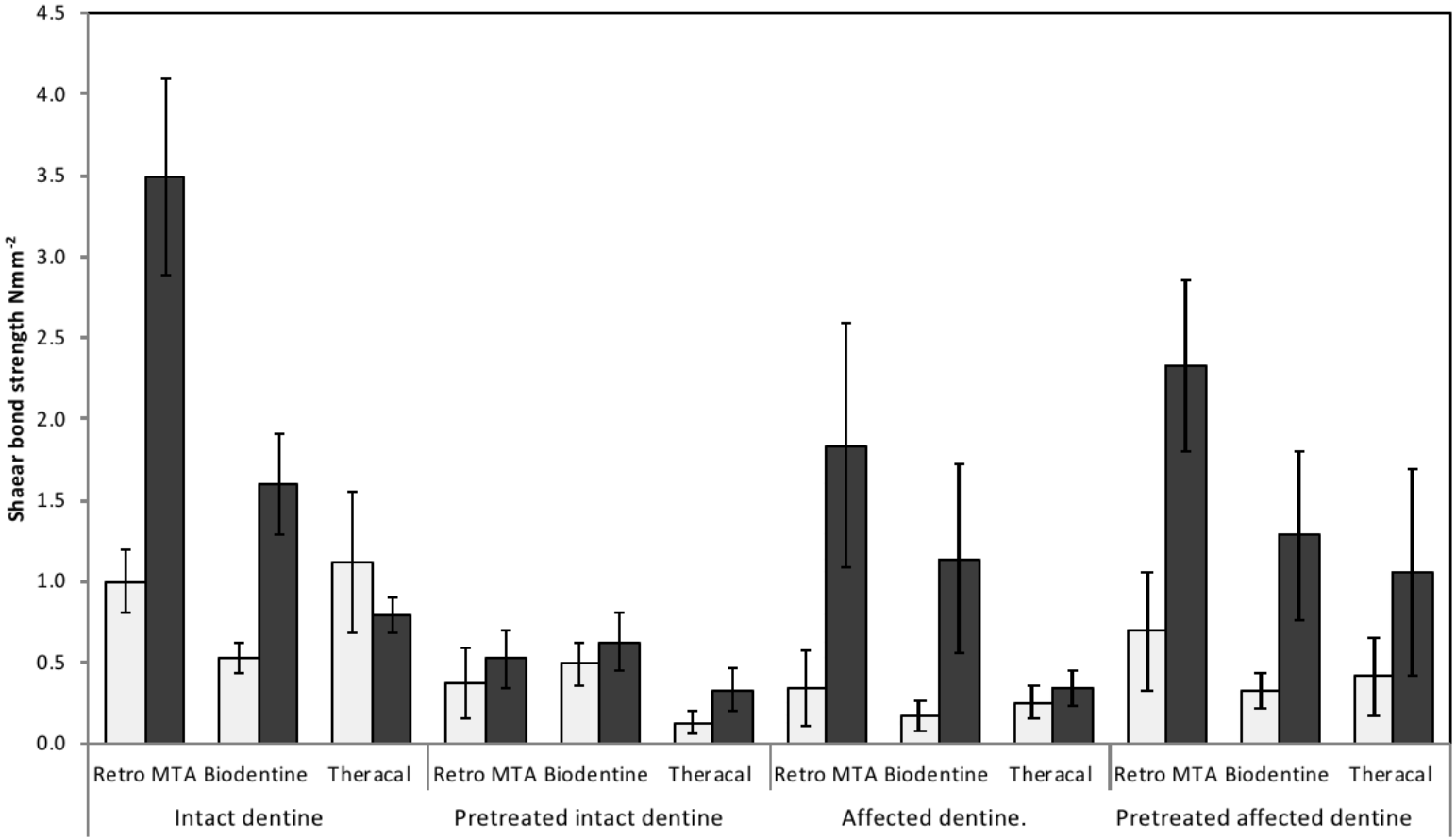
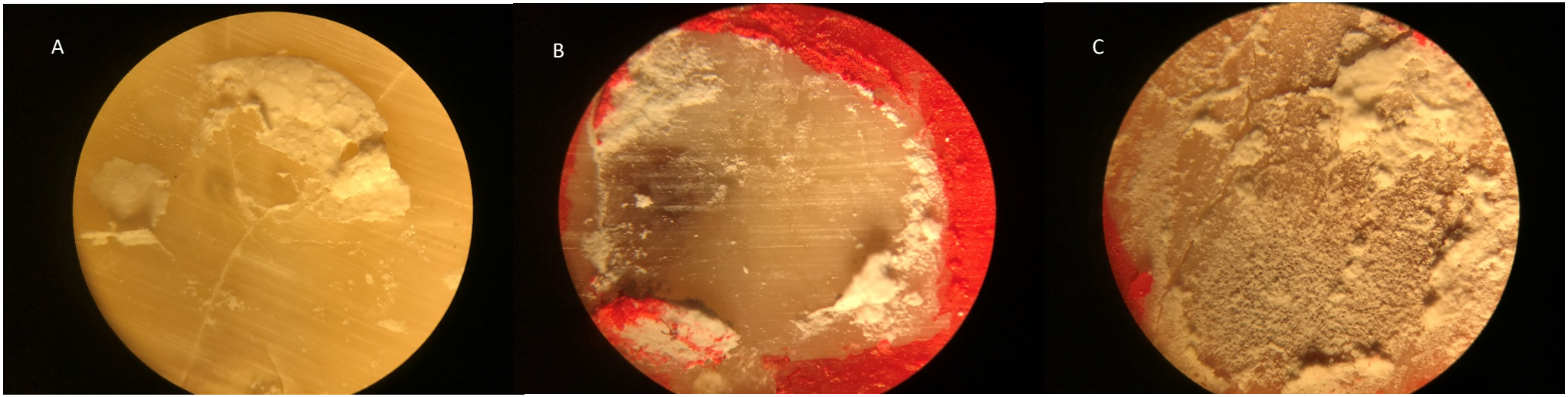
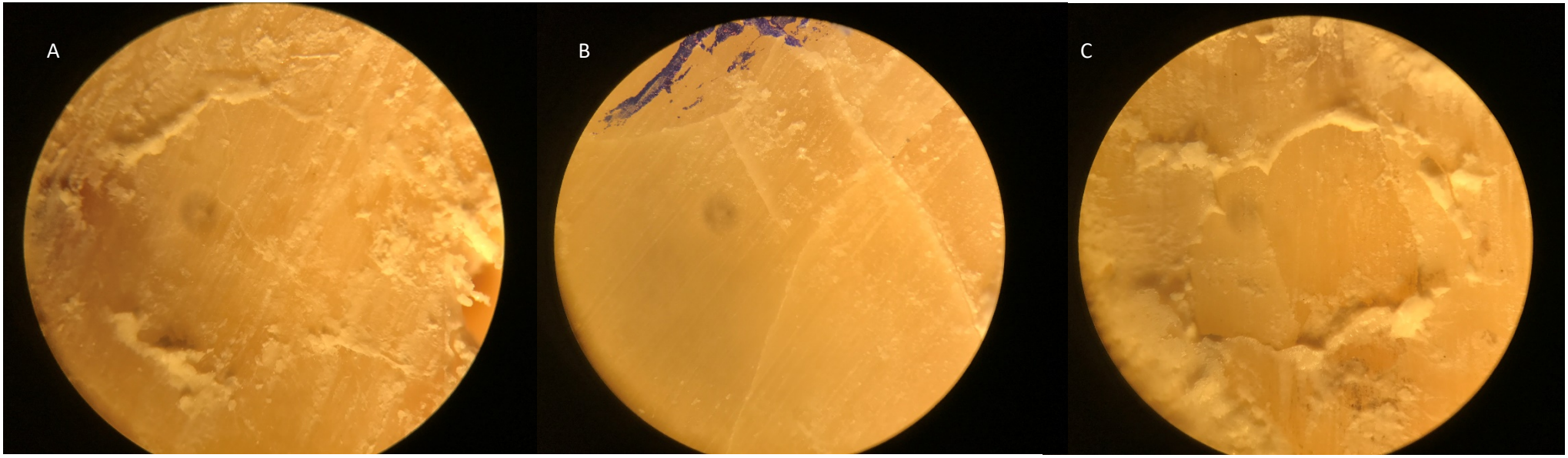


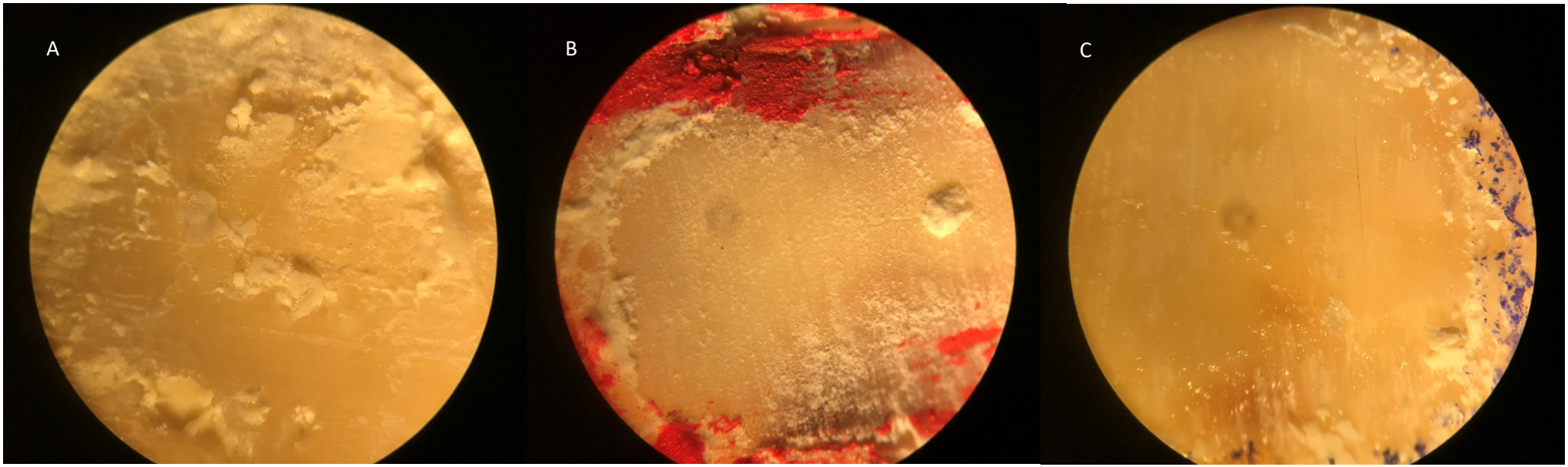
Figure 3a: Results of shear bond strength of test materials to different dentine substrates ± SD



Retro MTA



Biodentine



Theracal

Figure 3b: Images of failed specimens after shear bond strength testing. A: Intact dentine; B: Affected dentine and C: Affected dentine after pretreatment with NaOCl showing mixed failure modes

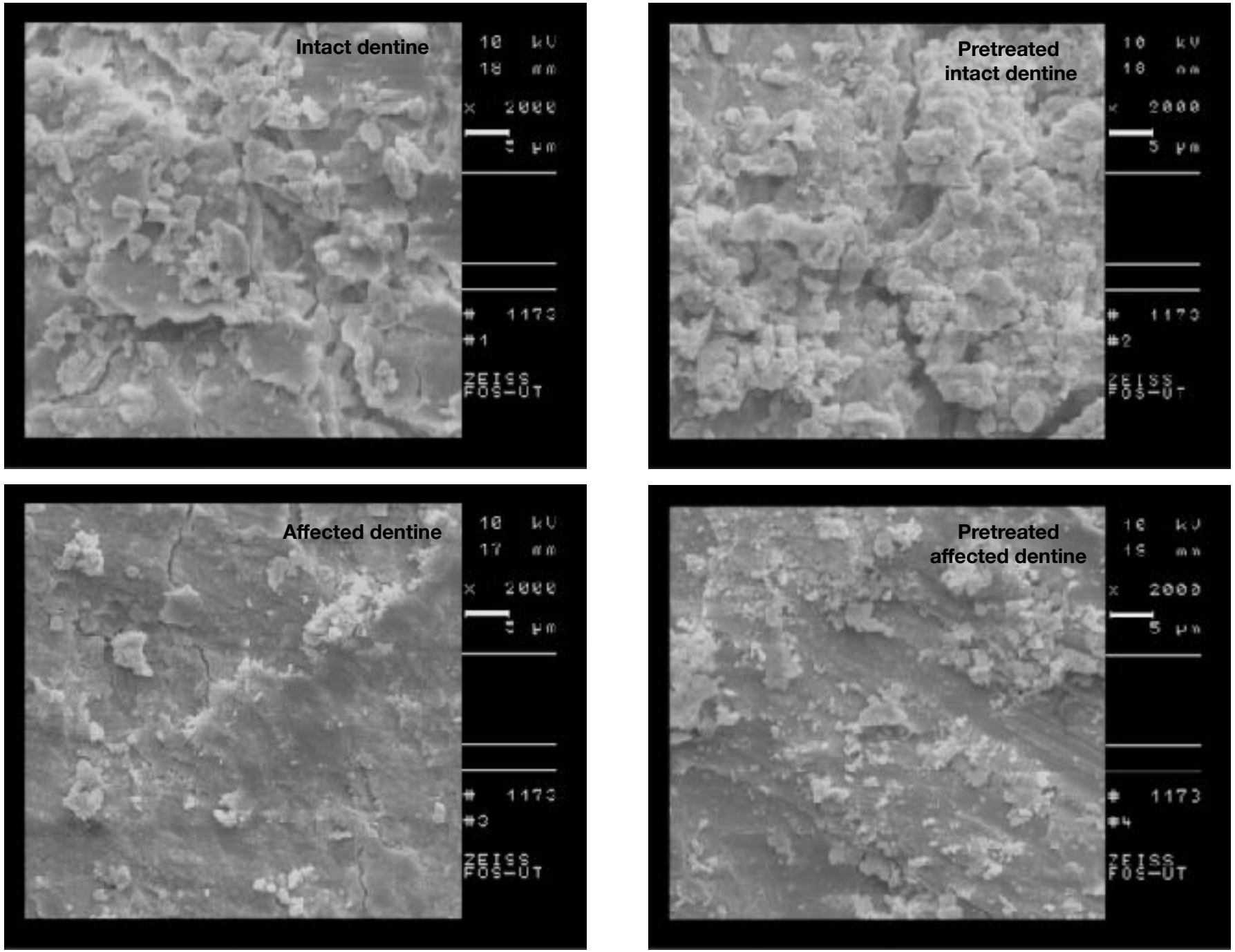


Figure 4a: Scanning electron micrographs of the different types of dentine used in the experiment (mag. 2Kx)

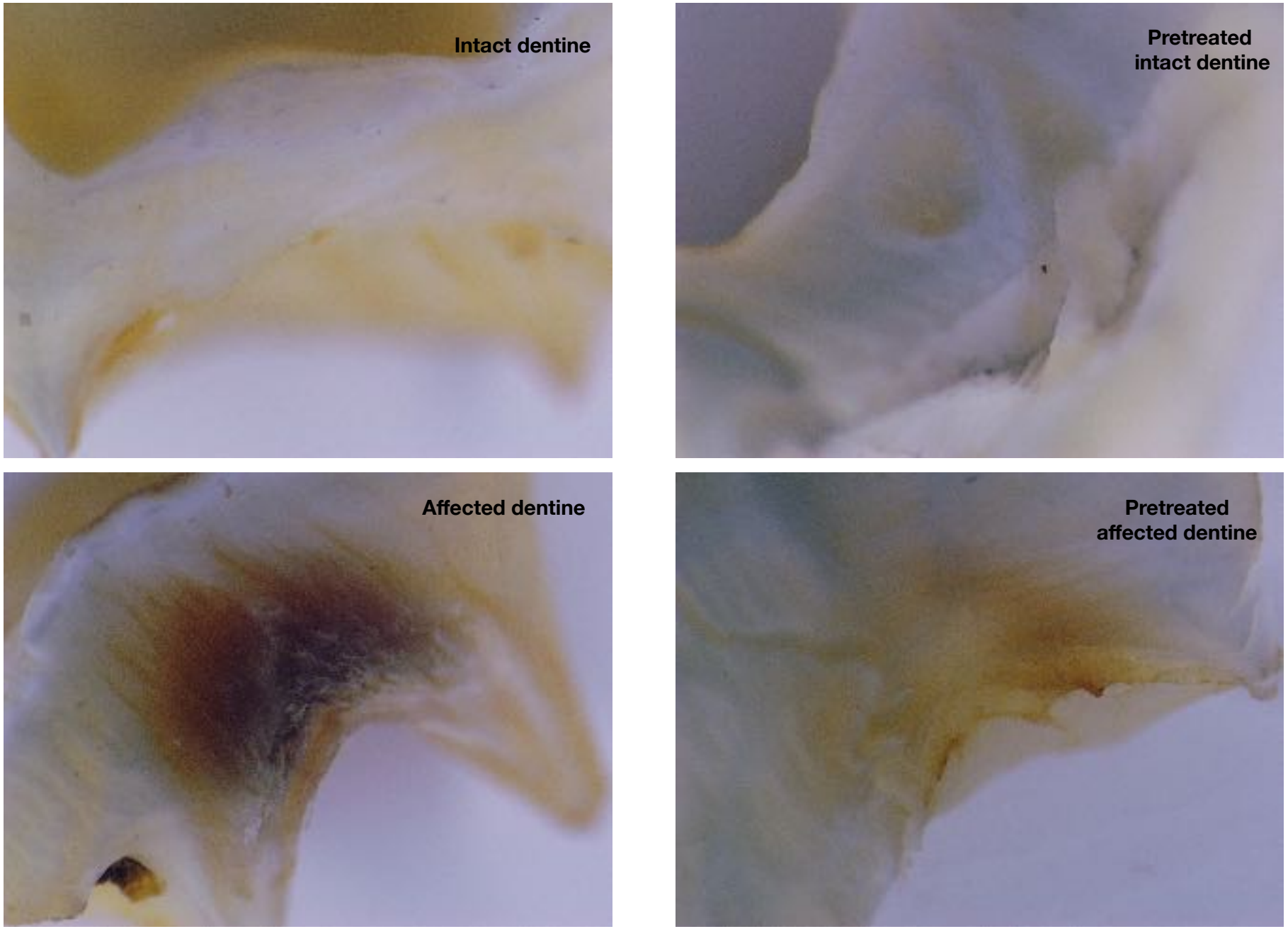


Figure 4b: Stereo-microscopic images of the different types of dentine used during the experimental procedures

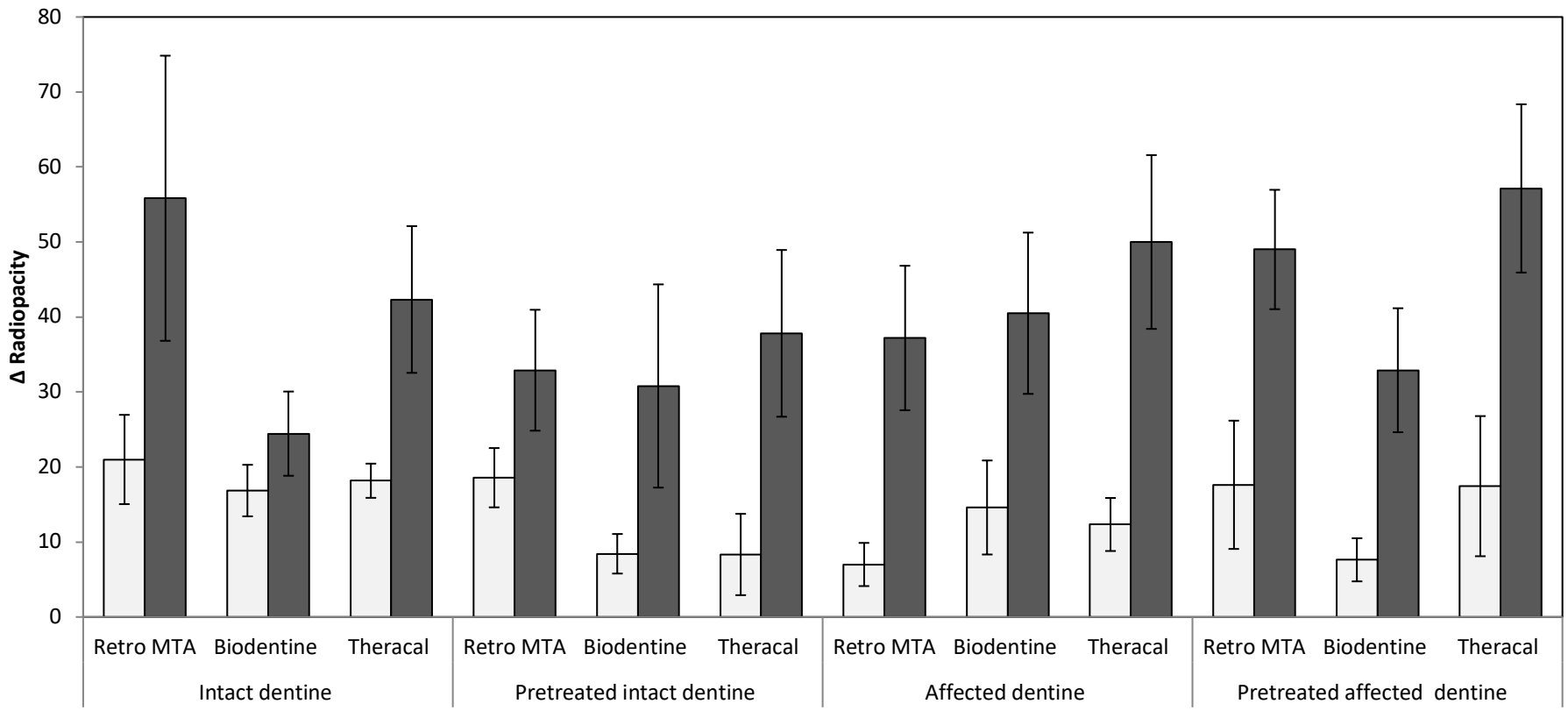


Figure 5: Changes in radiopacity of the dentine blocks over time when using the test materials